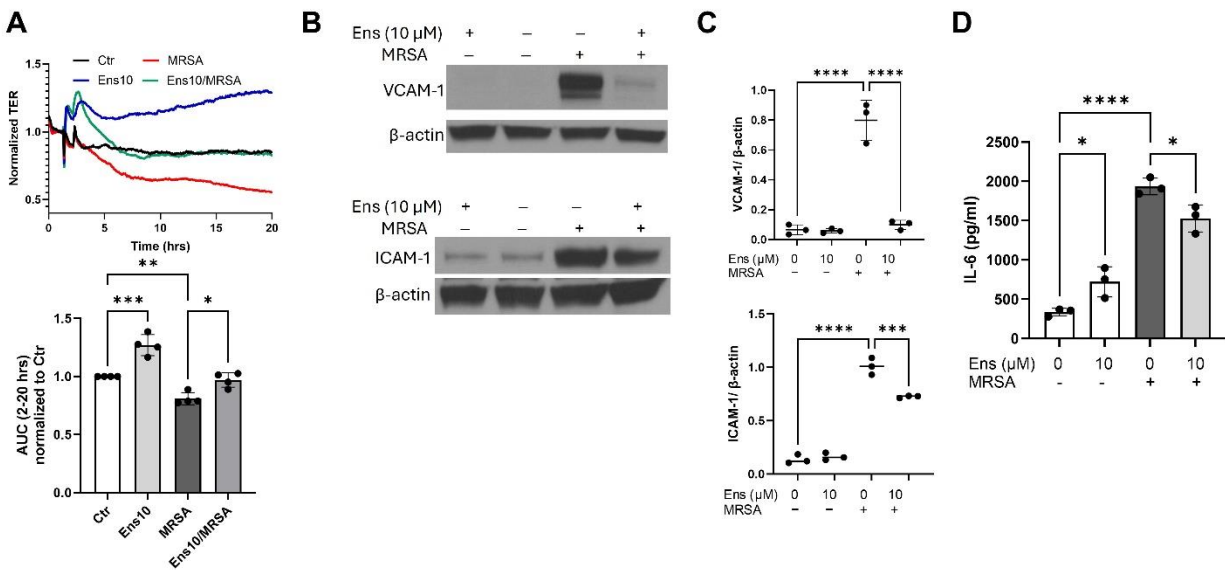
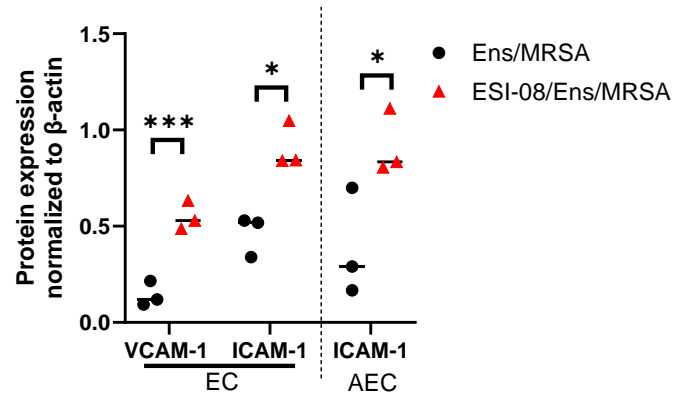


Supplementary Figure 1. Early phase effects of ensifentrine on EC barrier function. Human pulmonary artery endothelial cells (HPAEC) were treated with ensifentrine (5 μ M) or vehicle (DMSO). EC barrier function was assessed using the ECIS assay. Depicted is a representative plot of normalized TER values recorded for 1 hour after addition of ensifentrine.



Supplementary Figure 2. Effects of ensifentrine (10 μ M) on lung EC barrier function after MRSA treatment. HPAEC were pre-treated with ensifentrine (10 μ M) or vehicle (DMSO) for 1 hour prior to HK-MRSA challenge (2.5×10^8 /ml). EC barrier was assessed with the ECIS assay. **(A)** Representative TER tracings over time. Data were quantified after calculating the area under the curve for each condition. **(B-C)** Representative western blots of EC lysates for VCAM-1 and ICAM-1 expression and corresponding densitometry. **(D)** IL-6 levels were measured in EC

supernatants. N=3-4 independent experiments. Data were analyzed using one-way Anova, * $p<0.05$, ** $p<0.01$, *** $p<0.001$, **** $p<0.0001$.



Supplementary Figure 3. EPAC antagonism by ESI-08 alters the effects of ensifentrine on adhesion molecule expression in lung EC and AEC exposed to MRSA. HPAEC and A549 were pre-treated with 20 μM or 5 μM ESI-08 (EPAC antagonist) respectively. After 1 hour, ensifentrine was added (5 μM for HPAEC and 15 μM for A549). Cells were treated with HK-MRSA 1 hour later ($2.5 \times 10^8/\text{ml}$, 20 hrs), and cell lysates were analyzed for VCAM-1 and ICAM-1 expression. Depicted is the densitometry analysis. Data were analyzed using t-test, * $p<0.05$, *** $p<0.001$.